

IT IS CLAIMED:

1. A method of generating tethered extracellular or intracellular domains of transmembrane proteins comprising:

5 (a) preparing an operable expression vector consisting of a 5' signal sequence, a purification epitope tag, a sequence coding for the extracellular domain of a membrane protein, and a 3' anchor sequence; and

transfecting mammalian cells in vitro with said expression vector to generate an anchor tethered protein targeted to the extracellular domain of a plasma membrane; or

10 (b) preparing an operable expression vector consisting of a 5' myristoylation encoding sequence, a sequence coding for the intracellular domain of a membrane protein, and a 3' purification epitope tag; and

transfecting mammalian cells in vitro with said expression vector to generate a myristoylated tethered protein targeted to the intracellular domain of a membrane.

15 2. The method according to claim 1, wherein said 3' anchor sequence is a GPI anchor sequence.

20 3. The method according to claim 2, wherein said GPI-anchor sequence comprises the 32 terminal amino acids of the GPI-anchoring sequence.

4. The method according to any previous claim, wherein said mammalian cells are selected from the group consisting of CHO or HEK-293 cells.

25 5. The method according any previous claim, wherein said signal sequence is selected from a protein selected from the group consisting of epidermal growth factor, insulin, nerve growth factor, platelet-derived growth factor, glucagon, ICAM-1, B7-1, TrkA, platelet-derived growth factor receptor, and CD58.

30 6. The method according to any previous claim, wherein said purification epitope tag is a hexa-histidine epitope tag.

7. The method according to any previous claim, wherein said myristoylation-encoding sequence is a c-Src myristoylation-encoding sequence.

8. An operable expression vector for generating a tethered extracellular domain protein consisting of:

- a 5' signal sequence,
- a purification epitope tag;
- 5 a sequence coding for the extracellular domain of a membrane protein; and
- a 3' anchor sequence.

9. The vector according to claim 8, wherein said anchor sequence is a GPI sequence.

10 10. The vector according to claim 8 or 9, wherein said purification epitope tag is a hexahistidine epitope tag.

11. An operable expression vector for generating a tethered intracellular domain protein consisting of:

- 15 a 5' signal sequence for myristoylation;
- a sequence coding for the intracellular domain of a membrane protein; and
- a 3' purification epitope tag.

12. The vector according to claim 11, wherein said purification epitope tag is a hexahistidine epitope tag.

13. The method according to any one of claims 1 to 7 further comprising:
purifying said anchor tethered protein.